



# Cross Brain–Gut Analysis Highlighted Hub Genes and LncRNA Networks Differentially Modified During Leucine Consumption and Endurance Exercise in Mice with Depression-Like Behaviors

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## Abstract

Depression is a frequent mood disorder that might impair the brain-gut axis. In this study, we divided 30 mice into five groups: untreated mice, mice with depression-like behaviors, mice with depression-like behaviors treated with consumed leucine, mice with depression-like behaviors treated with exercise training, mice with depression-like behaviors treated with exercise training along with consumed leucine. According to artificial intelligence biological analysis, we found some mediators such as lncRNAs profile and *Kdr/Vegfa/Pten/Bdnf* interactions network in the hippocampus region and ileum tissue which could be decisive molecules in the brain-gut axis. Moreover, KDR as a principal cutpoint protein in the network was identified as the pharmaceutical approach for major depressive ameliorating based on pharmacophore modeling and molecular docking outcomes. Furthermore, we indicated that the mRNA and protein level of the *Pten* enhanced and *Vegfa/Kdr/Bdnf* mRNAs, as well as the protein level of KDR, decreased in mice with depression-like behaviors. Moreover, exercise and leucine ameliorated the brain-gut axis in mice with depression-like behaviors. Exercise and leucine regulated the lncRNAs network in the hippocampus and ileum of mice with depression-like behaviors. We suggest that the lncRNAs profiles could be considered as diagnosis and prognosis biomarkers, and exercise + leucine might be a practical approach to improve depression.

**Keywords** Endurance exercise · Leucine · Depression · LncRNAs · Brain-gut axis

## Background

In developing and developed countries, psychological disorders have imposed heavy economic and social burdens on health care systems [1, 2]. Depression is a frequent mood disorder that could lead to increased cerebrovascular disorders, cardiac diseases, gastrointestinal diseases, disruption of

microbiota in the ileum, other medical causes of mortality, and the risk of anxiety and depression speculation during the coronavirus pandemic [3–5]. Although the pathomechanism of depression is not entirely elucidated, an artificial intelligence survey indicated that several signaling pathways such as neuroinflammation, neurotransmitter functions, phosphatidylinositol signaling system, gap junction, muscle contraction, insulin signaling pathway, and dopaminergic synapse could be involved in this pathogenesis [5, 6].

Growing evidence has indicated various neurohumoral transmissions enabling the communication between the gut and central nervous system (CNS). Also, there are mutual interactions between the mucosal immune system and enteric nervous systems, and this cross-talk is called the gut-brain axis [7–9]. The gut-brain axis could modulate behavior, mucosal immune system, and emotion [10]. Hence, the gut function is essential for healthy brain function [11]. Based on the evidence, the gut microbiota is a critical environmental compound that affects gastrointestinal system function [12]. Notably, microflora could regulate human health and illness by bidirectional communication

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between the microbiota and the host [13]. Interestingly, the consumption of antibiotics could deplete the gut microbiota, disarrange intestinal epithelium, disrupt the expression of neuromodulators, and lead to cognitive disability in mice [14]. The gut microbiota might interact with genetic variables to co-regulate illness symptoms in genetically vulnerable people [7].

Furthermore, immense evidence has revealed that depression impairs the intestinal bacterial community, increasing inflammation in the intestinal epithelium and disrupting the gut-brain axis [15]. Thereby, the gut function is essential for healthy brain function [16, 17]. However, the mechanistic and molecular pathway is not elucidated. Recent evidence has indicated that the gut-brain axis had a critical role in digestion and absorbance via regulating microbiota in the intestinal epithelium [18].

On the other hand, Darren W. Roddy et al. revealed that the hippocampus area size in depressive conditions was reduced compared with healthy individuals. Moreover, they have shown a direct association between hippocampus size and depression stages [19]. Hence, the hippocampus might play a decisive role in the brain-gut axis.

There is no substantial treatment strategy to halt the progress of depression. Notably, only 60% of depression treatments reported improved symptoms [18]. Therefore, considering effective treatment accompanied without any adverse effect could provide insight into diminishing this disorder and ameliorating the ecosystem in the gastrointestinal system. In addition, based on the epidemiologic evidence, environmental agents might play crucial roles in depression [20]. Therefore, current studies focus on physical activity and dietary interventions in managing depressive symptoms [21].

Moreover, it was well-established that sufficient physical activity and nutrients could be requirements for producing neurotransmitters [22]. Physical activity has been reported to have a relationship with reduced risk of depression [23, 24]. Exercise is considered a non-pharmacological intervention that could be a valuable tool to halt and manage the onset of depression [25]. In addition, physical activity, possibly through positive effects on self-efficacy, coping strategies, quality of life, and body image, caused decreasing mental disorders [26].

Interestingly, aromatic amino acids, including tyrosine, phenylalanine, and tryptophan, could benefit by producing neurotransmitters in therapeutic depression [27, 28]. Based on recent evidence, leucine competed with aromatic amino acids for transport across the blood–brain barrier (BBB) and also could trigger a delay in serotonin secretion, suppression of central fatigue, and decreased level concentration of aromatic amino acids, which led to the possibility of reduced neurotransmitters related to aromatic amino acids. However, although the concentration of leucine significantly declined

in depressive conditions, the pathomechanism of this phenomenon is not clear [29].

Recently, evidence has suggested that compared with protein-coding genes in body tissues and fluids, lncRNAs (long non-coding RNAs), roughly more than 200 nucleotides, are feasible to be identified via various techniques, have higher stability in body tissues and fluids, and also have specific expression patterns in tissue [7]. Furthermore, growing evidence has emerged showing that lncRNAs could modulate the expression level of genes through different molecular mechanisms [30]. Therefore, there are several possibilities in lncRNA function associated with the various diseases such as lncRNAs that might influence the neighboring genes that regulate the expression level of networks and could be affected by SNPs [31]. In this artificial system biology study, the principal aim was to evaluate the decisive lncRNAs and hub genes in depression and also demonstrate that the route network between the brain and intestine might improve with regular exercise and consumption of leucine.

## Materials and Methods

### Bioinformatics Analysis

To predict the master genes involved in the brain-gut axis, we analyzed the gene expressions between two microarray datasets (GSE151807 and GSE171275) with major depressive disorders in various brain regions [32]. Moreover, we engaged GSE64004 in ileum tissue suffering from depression and stress using the Bioconductor package in R statistical programming language. These datasets were analyzed using the Mouse430\_2 and Mouse Gene 2.1 Affymetrix Array platform, collaborating with Oligo and Limma packages. Significant differential gene expressions obtained from R statistical analysis of microarray datasets (brain and ileum) separately were illustrated on the HeatMap diagram ( $P < 0.001$ ). We limited these genes based on  $P < 0.01$  and  $\log_{2}FC \pm 0.2$  cut off to determine essential hub genes among the list of the genes collected from the dataset analysis. Hence, we engaged 1308 and 1003 genes as significant differential gene expressions in the hippocampus and ileum in major depressive conditions. The protein–protein interaction networks constituted were drawn based on the medium confidence threshold in the STRING 11.0 database [33].

Furthermore, calculating essential network parameters such as degree, betweenness, and closeness was utilized by Cytoscape 3.6.0 software [34]. By applying network parameters, we obtained 120 hub genes from ileum tissue analysis based on betweenness: 0.006, closeness: 0.259, and degree: 9. Moreover, 112 hub nodes were detected from analysis of significant gene expressions of hippocampus tissue by considering betweenness:0.012, closeness: 0.175, and degree:

6. To remarkable pivotal molecular and cellular signaling pathways involved in the mood disorder pathomechanism, molecular networks were investigated in specific biomedical servers, a KEGG pathway analysis as a sub-browser of the KOBAS-i database, by  $P < 0.05$  [35]. To determine the common genes between the two hub node lists, we used the Venny 2.1.0 tool [36]. Based on the interactive tool results, 46 overlapped hub genes were concluded. A protein–protein interaction network including 46 overlapped hub genes based on network parameters and eigenvector centrality revealed that four genes, *VEGF- $\alpha$*  (vascular endothelial growth factor A), *KDR* (vascular endothelial growth factor receptor 2 or kinase insert domain receptor), *PTEN* (phosphatase and tensin homolog), and *BDNF* (brain-derived neurotrophic factor), having the highest degree and most betweenness, could be affected by other hub gene expression patterns under the influencing of endurance exercise and leucine consumption. Enrichment of 46 hub proteins based on KEGG, Panther [36], and KOBAS-i algorithms highlighted significant molecular signaling pathways while considering  $P < 0.05$ .

### lncRNA Prediction

To determine significant lncRNAs with differential transcription profiles in depression, we browsed the gene expression omnibus database and selected GSE189233 for analysis. On the other hand, in this in silico study, we predicted long non-coding RNAs related to VEGF $\alpha$  and PTEN based

on diseases and target genes by LncTard [37], LncRNADisease, mammalian ncRNA-disease repository (MNDR), and LncBook servers. Considering the expression levels of predicted lncRNAs in the ileum tissue and hippocampus region in the LncSEA database, GAS5 (growth arrest specific 5), HOTAIR (HOX transcript antisense RNA), MEG3 (maternally expressed 3), and TUG1 (taurine upregulated 1) were selected. Next, we showed that four long non-coding RNAs had an overlap between *PTEN* and *VEGF- $\alpha$* . In Fig. 1, we depicted the workflow of the study.

### Drug Design

Based on evidence and visualization of networks between selected hub genes for experimental assays, VEGFR2 (*KDR*) as a pivotal receptor has been involved in initiating molecular signaling pathways. Also, we realized that VEGFR2, as a potential cut point with a pivotal role in molecular and cellular pathways, could be effective in other expression profiles of genes involved in the network. Therefore, we obtained VEGFR2's ligands from Binding Database for pharmacophore model design. Moreover, the three-dimensional structure of hub proteins was downloaded from the Protein Data Bank server [38], and the structure-data file (SDF) of leucine was stored from the PubChem database [39]. We investigated leucine's pharmacokinetic and pharmacodynamic features based on the Swiss ADME database [40]. We indicated that leucine was a necessary complement with high absorbance in the gastrointestinal system, and with

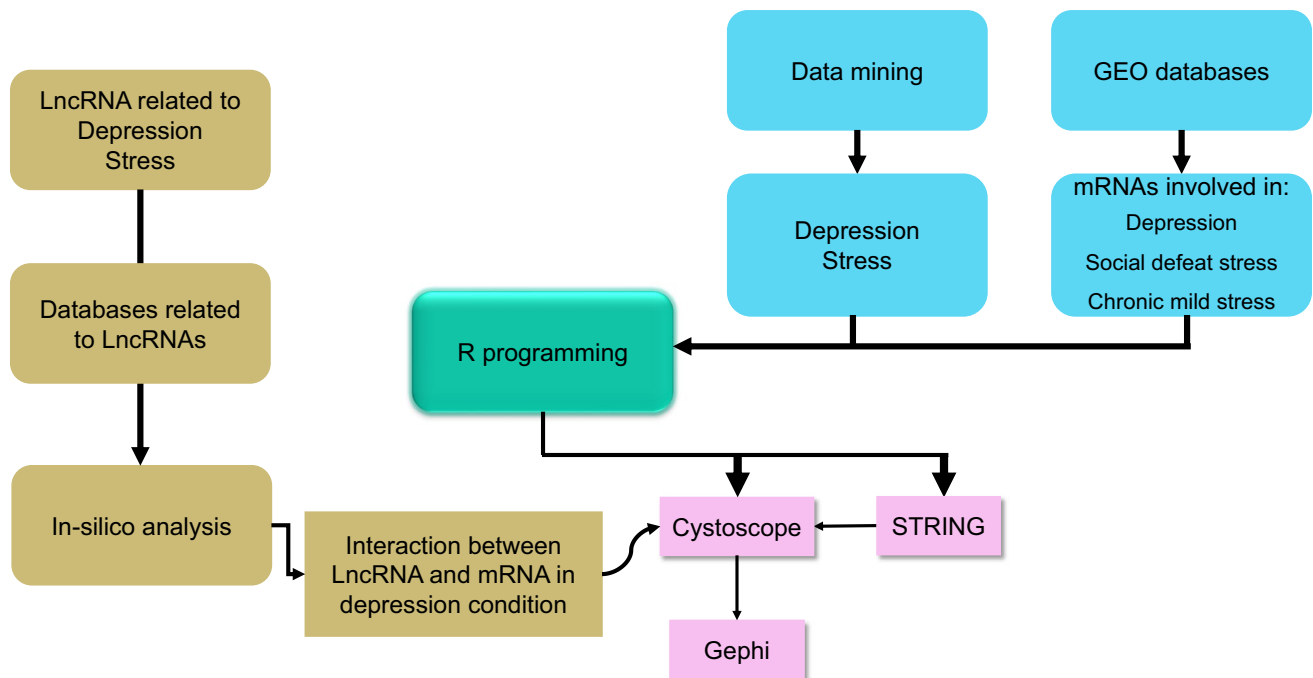


Fig. 1 In silico machine flowchart of the study

lipophilicity feature  $\text{Log } P = 1.14$  and topological polar surface area (TPSA) =  $63.32 \text{ \AA}^2$ , it could cross the blood–brain barrier (BBB). According to these results, we hypothesized that leucine consumption could affect the gene expression profile of the ileum and the brain.

Thus, we designed a suitable pharmacophore model based on active ligands obtaining the binding database library. First, the pharmacophore model design was performed based on the OPLS-2005 force field option by Ligprep and Phase-Pharma applications with 4 to 5 features and 80% of active ligands ( $\text{IC}_{50} < 1$ ) in the Schrödinger server [41]. Next, we tried matching the leucine structure as a ligand on the pharmacophore model built. Moreover, we computed the leucine's binding affinity and stability on VEGFR2, VEGF- $\alpha$ , PTEN, and BDNF by molecular docking method in PyRx software [42]. This calculation made a contract based on binding affinity  $< -5$  and root-mean-square deviation of atomic positions (RMSD)  $< 2$  as suitable binding energy between macromolecule and small molecule. Then, optimization and preparation of three-dimensional structures of macromolecules were performed following extra chains ablation and removing ligands and non-complex compounds, based on dock prep tools in Chimera 1.8.1 software [43]. Finally, molecular docking of VEGFR2, VEGF- $\alpha$ , BDNF, and PTEN was applied in the search space box with central dimensions: X: 65.9723, Y: 100.6747, Z: 35.8619 for VEGFR2; X: 12.6238, Y: 3.7757, Z: 7.9480 for VEGF- $\alpha$ ; X: 9.6256, Y: 19.4432, Z: 8.6052 for BDNF; X: 33.7273, Y: 83.7684, Z: 31.7349 for PTEN.

## Ethical Issue

All procedures were conducted and approved following the Research Ethics Committees of Islamic Azad University Isfahan (Khorasgan) Branch (IR.IAU.KHUISF.REC.1400.160).

## Animal Study

The 6-week-old male C57BL/6 mice were provided and housed at the animal house of the Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran. Mice were kept under a standard condition of  $24 \pm 3 \text{ }^\circ\text{C}$  temperature, 55 to 60% humidity, and 12-h light and dark cycles and fed normal diets and ad libitum water. Moreover, all animals fed a normal diet (contained 15% (w/w) fat, 58% (w/w) carbohydrate, 27% (w/w) protein, ad libitum, 3.2 kcal/1 g).

Mice were adapted for 1 week prior to the start of experiments. Mice with an approximate weight of  $23 \pm 2 \text{ g}$  were randomly assigned into five groups ( $n = 6$  per group): (control) untreated mice, (depression) mice

with depression-like behaviors, (Leu) mice with depression and consumed the leucine complement, (EXr) mice with depression with exercise training, (Leu + EXr) mice with depression with exercise training along with consumed leucine complement. The calorie intake, weight, and water drinking were monitored weekly. Mice fasted for 6 h before euthanasia, after 8 weeks of consuming the leucine complement and exercise training. Mice were euthanized by administration of ketamine (80 mg/kg body weight per mouse) and xylazine (10 mg/kg body weight per mouse).

## Inducing Depression

This study induced depression in all groups except untreated mice (control). Based on previous evidence, mice were depressed following protocols for 14 days [44, 45]. Subsequently, we divided depression mice into four groups, as mentioned above. First, mice were exposed to the electric tail and foot shock for 1 s with repetition ten times (intensity, 0.5 mA), deprivation of food, and fixation randomly conducted for 14 days [46, 47]. After that, the behavior test, including the open-field test, elevated plus maze test, tail suspension test, and social interaction test, was conducted to validate depression features. Then, we assessed the effect of the exercise training and consumed the leucine complement on the mice with depression-like behaviors.

## Mice Behavioral Tests

### Open Field Test

The open-field test was conducted on days 16 and 17. Moreover, the resting times and locomotor activity were measured. The open-field area was ( $40 \times 40 \times 40 \text{ cm}$ ), divided into 16 squares. Each mouse was placed in the central area and monitored for 1-h sessions. Distance moved (cm), duration time (s), and rest times (s) were scored and calculated [44, 45].

### Elevated Plus Maze Test

For evaluating the levels of depression and anxiety, this test was used. This device contained four arms with two opposite closed arms ( $35 \times 10 \times 1 \text{ cm}$ ) and open arms ( $35 \times 10 \times 1 \text{ cm}$ ). Each mouse was placed in the central area. In this study, the behavior was monitored for 1 h. In addition, the time spent in open and closed arms was recorded, and the number of

entries into each arm was recorded. Enhancing the number of entrances into the closed arm and time spent in the closed arm as described was considered to measure depression and anxiety [45].

### Social Interaction Test

We evaluated the social interaction using an open-field apparatus. Moreover, we used two plastic chambers placed on both sides of the box. In one of them, we placed a wild-type mouse (as a target) with no prior contact with the depressed mouse, and another chamber was empty (no target). Then, we placed the depressed mouse on the middle open-field apparatus and allowed it to freely explore in the box for 15 min (we considered 10 min a habituation trial). We calculated the amount of time spent around each chamber (wild-type mouse or empty) in this test. Total distance moved (cm) with no target, time spent in interaction zone (s) with no target, total distance traveled (cm) with the target, and time spent in interaction zone (s) with the target were measured and scored [45, 48].

### Tail Suspension Test

The mouse was stuck approximately 1 cm by an attached adhesive tape on its tail, and the behavior was assessed for 6 min. In this test, mice were allowed to adapt for 2 min. Total freezing was recorded as a depression-like behavior [45].

### Leucine Complement and Food Intake

Mice were fed with free access to standard food (contained 15% (w/w) fat, 58% (w/w) carbohydrate, 27% (w/w) protein, ad libitum, 3.2 kcal/1 g) and tap water. In addition, L-leucine (Catalogue Number: 105360, Merck) was injected (50 mg/kg, intraperitoneal injection) once per day for 8 weeks [45].

### Exercise Protocol

Endurance exercise training (EXr) was conducted on a motorized treadmill. The intense exercise was moderate-high on the treadmill for 2 months (6 days/week). After that, exercise duration and running speed gradually increased to reach ~75% VO<sub>2</sub> max (32 m/min) for 45 min. Moreover, the treadmill slope was considered to be 0% [49, 50].

### qRT-PCR

Total RNA was extracted from the hippocampus region and ileum tissue using TRIzol reagent (Sigma, USA). According to the manufacturer's instructions, cDNA synthesis was conducted with 1 µg of total RNA using a cDNA synthesis kit (TaKaRa, Japan). The qRT-PCR was conducted with CYBR Green (TaKaRa, Japan) using Corbet rotor gene 6000 (Qiagen, Australia). Detection of gene expression was evaluated according to the 2<sup>-ΔΔCT</sup> method. The relative expression of genes was calculated based on glyceraldehyde-3-phosphate dehydrogenase (Gapdh) expression levels. Primer sequences were ordered through the MicroGen company (South Korea), and their sequences are listed in Table 1.

### Quantitative Protein level (Western Blot Assay)

Hippocampus was lysed via TRI reagent (Thermo Scientific, 15,596–018, USA). Moreover, we loaded 30 µg of sample protein for the SDS-PAGE, and then protein bands were transferred to the PVDF membrane (Bio-Rad, 162–0176, USA). The primary antibodies used were rabbit KDR antibody (Abcam, ab11939, USA), anti-PTEN (ab32199, Abcam), and mouse anti-GAPDH antibody (Santa Cruz, sc-32233). Membranes were incubated with primary

**Table 1** Primer sequences

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Primer length	Annealing temperature (°C)
Bdnf	CCACTAAGATACATCATAGC	CAGAACAGAACAGAACCA	106	55
VEGF-α	GCTACTGCCGTCCGATTGA	ATGGTGATGTTGCTCTCTGA	163	58
Pten	GGAAGTAAGGACCAGAGACAA	CACCACACACAGGCAATG	287	56
Kdr	TGTATGGAGGAAGAGGA	CTGTTCTGGAGATAATGAC	81	56
MEG3	ACAAGCGACAAAAGAGGAT	ATGAACACGAGCACAGAT	159	53
HOTAIR	TTGCTCTCCTTATCATCC	ATTAGTGCCCTCAGTCC	132	51
GAS5	AGTGTGGACCTCTGTGAT	CTCGTAAGCCTTCATCCTCCT	78	51
TUG1	CTCTGGAGGTGGACGTTTTGT	GTGAGGTCGTGTCTCTCTTTTCTC	72	51
GAPDH	TGCCGCTGGAGAAACC	TGAAGTCGCAGGAGACAACC	121	58..6



antibodies for 2 h at room temperature, then for 1 h at room temperature with an appropriate secondary antibody. We detected each protein bandwidth Amersham ECL Advance Western Blotting Detection Kit (GE Healthcare, USA). Furthermore, the intensity of bands was calculated by ImageJ software.

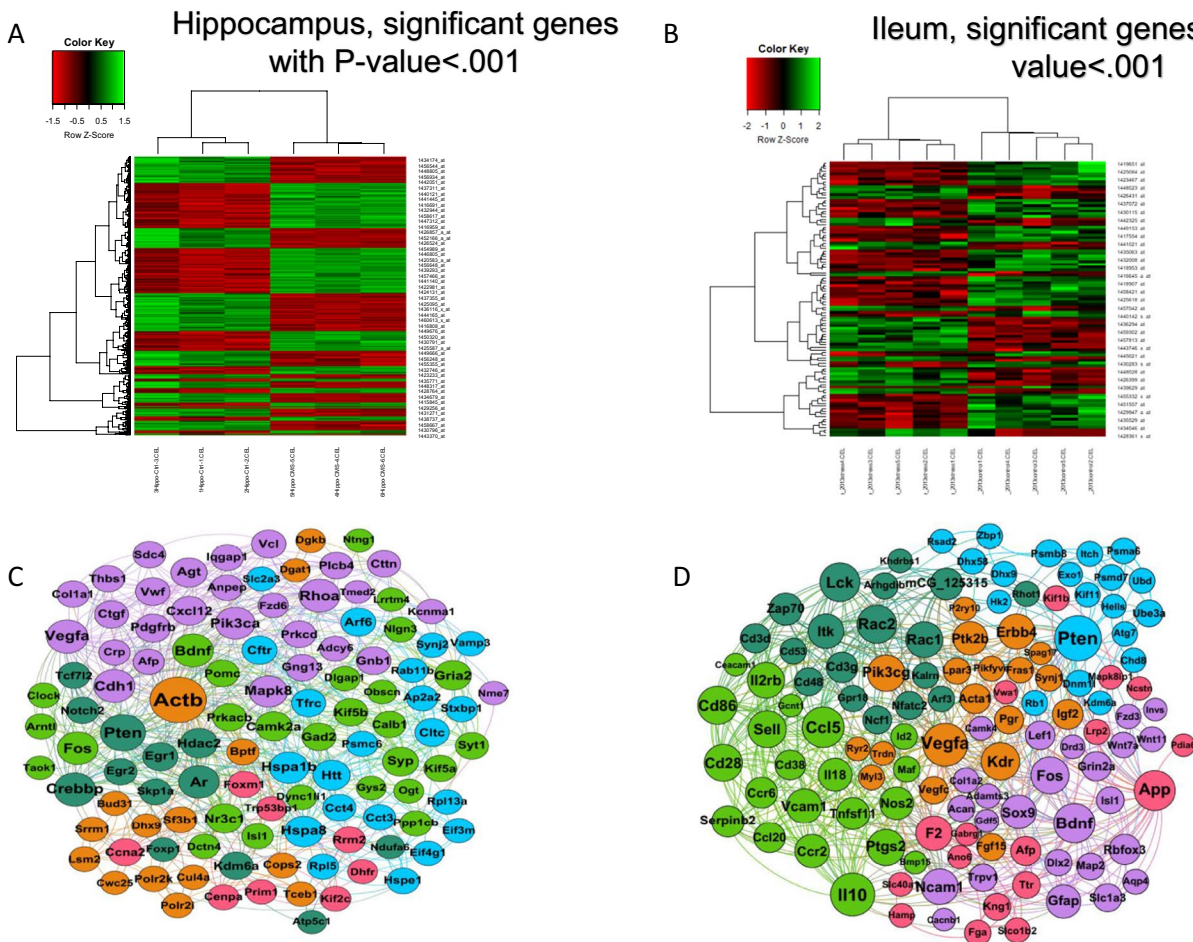
### Statistical Analysis

The sample size was estimated based on 80% power and an alpha level of 0.05. Statistical analysis was conducted using GraphPad Prism (Version 9; GraphPad Software). The Shapiro–Wilk test was used for normalizing distribution, and variables were normally distributed. Data were analyzed by one-way analysis of variance (ANOVA) with Tukey’s post hoc test due to multiple comparisons. Differences at  $P < 0.01$  were considered to be significant. Moreover, data were indicated as the mean  $\pm$  SD.

## Result

### Cross-talk Between Brain and Gut Axis: In Silico Machine

Based on R statistical analysis, we collected 1308 genes with differential expression in mice with major depressive disorders in the hippocampus region compared to normal tissue. Moreover, 1003 differential gene expressions were identified in the ileum tissue of depressive mice compared to normal mice. We showed these differential gene expressions via the HeatMap diagram in Fig. 2A and B, with  $P < 0.001$ . According to the optical network’s parameters, such as betweenness, degree, and closeness, we calculated the centrality parameters of hub genes. Next, we designed the protein–protein interaction network to consider the nodes’ degree, modularity, and betweenness centrality (Fig. 2C, D). Enrichment of hippocampus’s hub genes in KEGG pathway analysis as



**Fig. 2** Protein–protein interactions based on network visual parameters. **A, B** HeatMap diagram of differential gene expressions based on R statistical analysis with  $P < .001$  in the ileum and hippocampus of mice with depression-like behaviors. **C, D** Protein–protein inter-

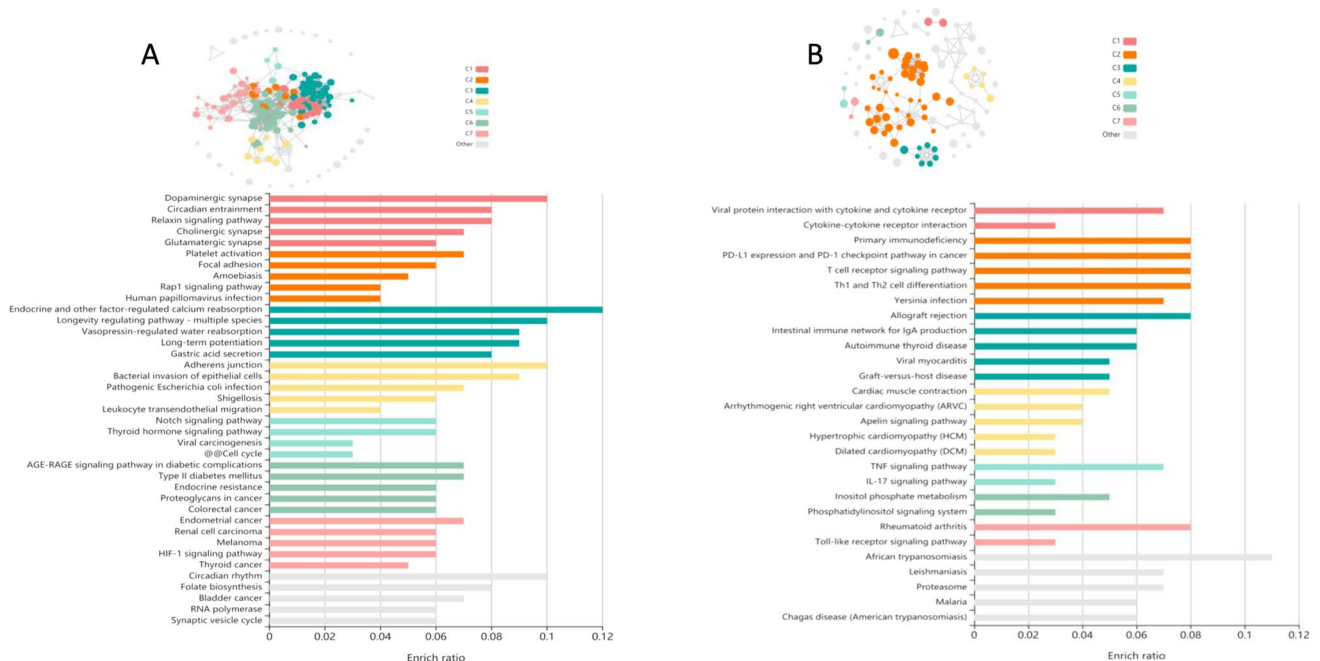
action network designed based on visual parameters of hub proteins with considering the degree, betweenness, and closeness in modularity class’s color and size for hub genes involved in the ileum and hippocampus in depression condition

sub-browser of the KOBAS-i server for determining significantly molecular signaling pathways with  $P < 0.05$  marked several signaling and molecular mechanisms such as dopaminergic synapse, platelet activation, focal adhesion, Rap1 signaling pathway, Notch signaling pathway, cell cycle, and AGE-RAGE signaling pathway involved in the hippocampus region with depressive disorders (Fig. 3A).

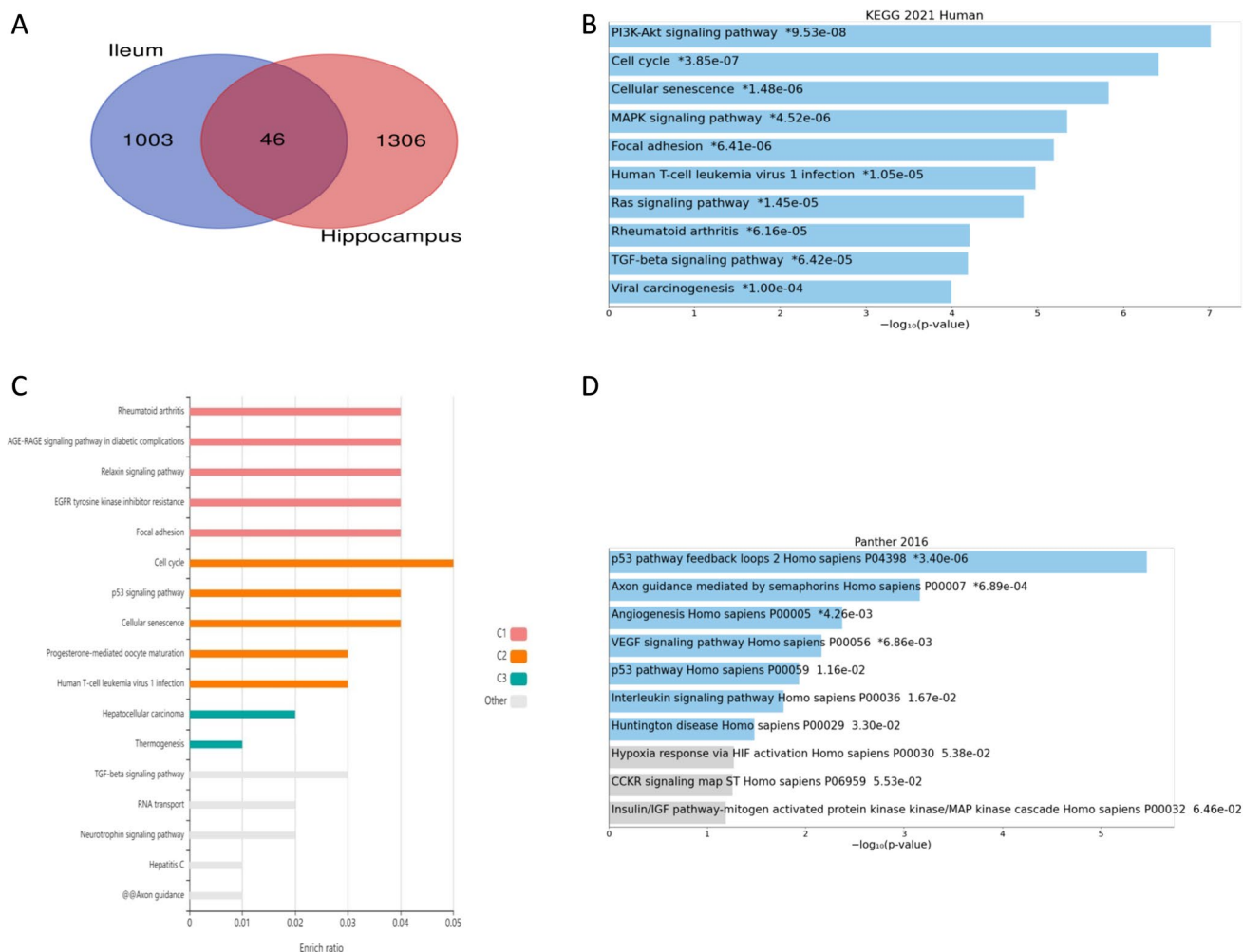
On the other hand, the bar graph of ileum's hub genes enrichment manifested cytokine-cytokine receptor interaction signaling pathway, immunodeficiency, TNF signaling pathway, IL-17 pathway, Toll-Like receptor, and cell differentiation as crucial molecular signaling pathways involved in the pathomechanism of the ileum tissue in depressive conditions (Fig. 3B). Moreover, based on the Venn diagram results, 46 common hub genes were distributed between the hippocampus region and ileum tissue (Fig. 4A). Based on the design protein–protein interaction network construction, we indicated that four genes could be pivotal in the brain-gut axis, including *Pten*, *Vegf- $\alpha$* , *Kdr*, and *Bdnf*. We selected these common genes via the critical network's highest betweenness and maximum degree. Significant molecular signaling pathways associated with common genes based on the enrichment analysis in the KEGG pathway, Wiki pathway, Reactome, and Panther have marked several pathways such as PI3K-AKT signaling pathway, AGE-RAGE signaling pathway, P53 signaling pathway, TGF-B signaling pathway, MAPK signaling pathway, RAS signaling pathway, and focal adhesion as vital pathways involved in the gut-brain axis (Fig. 4B–D).

## Interaction Between LncRNA and mRNA in Depression Condition

Integrative analysis of non-coding RNAs (LncRNAs) and coding RNAs (mRNAs) was performed in neurodevelopmental impairments in the gut dysbiosis mice based on GEO2R analysis. To specify significant LncRNAs,  $P < 0.05$  was applied, which highlighted 358 LncRNAs (195 overexpressed and 163 down expressed). Based on non-coding microarray analysis, GAS5 with downregulation pattern expression was confirmed in depression-like behavior in mice compared to healthy mice. Moreover, interaction types of LncRNAs-mRNAs were browsed in the LncRNAs databases and admitted by text mining (Table 2). On the other hand, the prediction of non-coding RNAs, especially long non-coding RNAs (lncRNAs) as regulators of the gene's expression, was performed based on genes and diseases. First, we prepared a list of LncRNAs related to selected genes (Table 3). Then, we confirmed the expression profiles of LncRNAs in the hippocampus region and ileum tissue based on the database survey and in silico analysis. Eventually, we specified four LncRNAs for expression measurement in two tissues (hippocampus and ileum) after regular exercise and consumption of leucine in depressive mice. Furthermore, we drew a comprehensive network between hub genes and selected LncRNAs that showed that four LncRNAs, including MEG3, HOTAIR, GAS5, and TUG1, are common between *Pten* and *Vegf- $\alpha$*  (Fig. 5A, B).



**Fig. 3** Hub proteins enrichment. **A, B** KEGG pathway enrichment of hub genes involved in hippocampus and ileum highlighted significant molecular signaling pathways in depression pathogenesis



**Fig. 4** Enrichment information of common hub proteins. **A–D** Significant molecular signaling pathways associated with common genes based on the enrichment analysis in the KEGG, KOBAS-i, and Pan-

ther algorithms have been marked principal paths as pivotal pathways involved in the gut-brain axis

## Leucine Complement as Psycho-Gut Therapy Drug Based on Pharmacological Modeling and Ligand Screening

Visualizing the network of selected genes involved in the PI3K-Akt signaling pathway in STRING and data mining led to the selection of KDR as a strong candidate for preventing and treating major depressive disorders and gut mucosal epithelium damage. Drug design with the pharmacophore modeling method was performed based on these data. The AHRRR pharmacophore model, including Acceptor, hydrophobic, and aromatic ring features, with the highest survival score of 5.762731 based on 251 active ligands ( $IC_{50} < 1$ ), was designed (Fig. 6A). In contrast to the designed pharmacophore model, the 3D structure of leucine did not match the model built, but the molecular docking technique with binding affinity  $-5.1$  kcal/mol and  $RMSD < 2$  confirmed the effect of leucine complement on

KDR (Fig. 6B). Furthermore, the drug design results based on the molecular docking method revealed that leucine with binding ability  $> -5$  kcal/mol and  $RMSD$  threshold  $< 2$  on BDNF, PTEN, and VEGF $\alpha$  proteins, due to the non-suitable binding affinity between leucine and the mentioned proteins, could not be influenced by the function of these proteins (Fig. 6C–E).

## Depression Reduced Mobility and Impaired Physical Features

This study found that mice with depression-like behaviors significantly gained weight more than did the control group (Fig. 7A, B:  $P < 0.01$ ). Moreover, we indicated that drinking water consumption and food intake were enhanced in mice with depression-like behaviors (Fig. 7C, D:  $P < 0.01$ ). Based on these physical features, we could conclude that the excessive food and water intake significantly increased compared



**Table 2** Text mining and browsing databases to determine the physiological function of lncRNAs in pathogenesis based on the targets

Disease name	Regulator	Target gene	Effect direction	Expression pattern	Regulatory mechanism	Function
Glioblastoma	TUG1	VEGF $\alpha$	Positive effect	Upregulation	ceRNA(miR-299)	Angiogenesis (+)
Hepatoblastoma	TUG1	VEGF $\alpha$	Positive effect	Upregulation	ceRNA(miR-34a-5p)	Cell growth (-) angiogenesis (-)
Malignant glioma	TUG1	PTEN	Positive effect	Upregulation	ceRNA(miR-26a)	Tumor-suppressive function (+)
Breast cancer	HOTAIR	PTEN	Negative effect	Upregulation	Histone modification	Cell proliferation (+) Cell invasion (+) Cell migration (+)
Laryngeal squamous cell carcinoma	HOTAIR	PTEN	Negative effect	Upregulation	DNA methylation	Oncogenic role (+)
Liver fibrosis	HOTAIR	PTEN	Positive effect	Upregulation	DNA methylation ceRNA(miR-29b)	Fibrotic (+)
Cervical cancer	HOTAIR	VEGF $\alpha$	Positive effect	Upregulation	Not reported	Cell motility (+) Cell metastasis (+)
Gastric cancer	HOTAIR	VEGF $\alpha$	Positive effect	Upregulation	Not reported	Chemoresistance (+) PI3K/AKT signaling pathway (+)
Hepatocellular carcinoma	HOTAIR	VEGF $\alpha$	Positive effect	Upregulation	Not reported	Cell proliferation (+)
Nasopharynx carcinoma	HOTAIR	VEGF $\alpha$	Positive effect	Upregulation	Not reported	Angiogenesis (+)
Cardiac fibrosis	GAS5	PTEN	Positive effect	Downregulation	ceRNA(miR-21)	Cell proliferation (-) Cell growth (-) fibrotic (-)
Endometrial cancer	GAS5	PTEN	Positive effect	Downregulation	ceRNA(miR-103a-3p)	Apoptosis process (+)
Hepatocellular carcinoma	GAS5	PTEN	Positive effect	Downregulation	ceRNA(miR-21)	Cell migration (-) Cell invasion (-)
Her2-receptor positive breast cancer	GAS5	PTEN	Positive effect	Downregulation	ceRNA(miR-21)	Chemoresistance (+) Cell proliferation (+)
Non-small cell lung cancer	GAS5	PTEN	Positive effect	Downregulation	ceRNA(miR-21)	Chemosensitivity (+)
Pancreatic cancer	GAS5	PTEN	Positive effect	Downregulation	ceRNA(miR-32-5p)	Cell metastasis (-)
Cervical cancer	GAS5	PTEN	Positive effect	Downregulation	Not reported	Chemoresistance (-)
Colorectal cancer	GAS5	VEGF $\alpha$	Negative effect	Downregulation	Not reported	NF- $\kappa$ B signaling pathway (-) ERK1/2 signaling pathway (-) Tumorigenesis (-)
Malignant glioma	MEG3	PTEN	Positive effect	Downregulation	ceRNA(miR-19a-3p)	Cell proliferation (-) Cell migration (-) Cell invasion (-)
Pulmonary hypertension	MEG3	PTEN	Positive effect	Downregulation	ceRNA(miR-21)	Cell proliferation (+) Cell migration (+)
Osteoarthritis	MEG3	VEGF $\alpha$	Negative effect	Downregulation	Not reported	Angiogenesis (-)

with the control group (Fig. 7C, D:  $P < 0.01$ ). In addition, we exhibited polydipsia and hyperphagia-like symptoms following an increase in weight and drinking water consumption. Here, we evaluated depression levels and the social defeat of the mice. Thus, we found that the distance moved (unite) and movement time (s) of mice with depression-like behaviors were reduced compared with the control group (Fig. 7E, F:  $P < 0.01$ ). In addition, the rest time of the mice with depression-like behaviors significantly increased with the control group (Fig. 7G:  $P < 0.01$ ). Furthermore,

the elevated plus-maze test revealed that the number of entries into the opened and closed arms had decreased and increased, respectively (Fig. 7H, I:  $P < 0.01$ ).

Moreover, the total movement of mice with depression-like behaviors significantly declined compared with the control group (Fig. 7J:  $P < 0.01$ ). Also, the freezing time of the mice with depression-like behaviors was increased compared with the control group (Fig. 7K:  $P < 0.01$ ). Furthermore, the social interaction test demonstrated that the total distance moved (cm) with no target, time spent in interaction zone

**Table 3** LncRNAs related to VEGF $\alpha$  and PTEN genes

Gene	LncRNA	
VEGF $\alpha$	MALAT1	
	HOTAIR	
	GAS5	
	MIAT	
	TDRG1	
	HNF1A-AS1	
	TUG1	
	H19	
	SNHG15	
	LINC00668	
	MEG3	
	HOXA11-AS	
	PTEN	PTENP1
		HOTAIR
		PTEN
		GAS5
CASC2		
BGLT3		
FER1L4		
AFAP1-AS1		
LNCARSR		
NORAD		
XIST		
LINC00161		
LNCRNA-ATB		
HULC		
TP53COR1		
TUG1		
MEG3		
OIP5-AS1		
HIF1A		
DLX6-AS1		
CYTOR		
RP11-79H23.3		

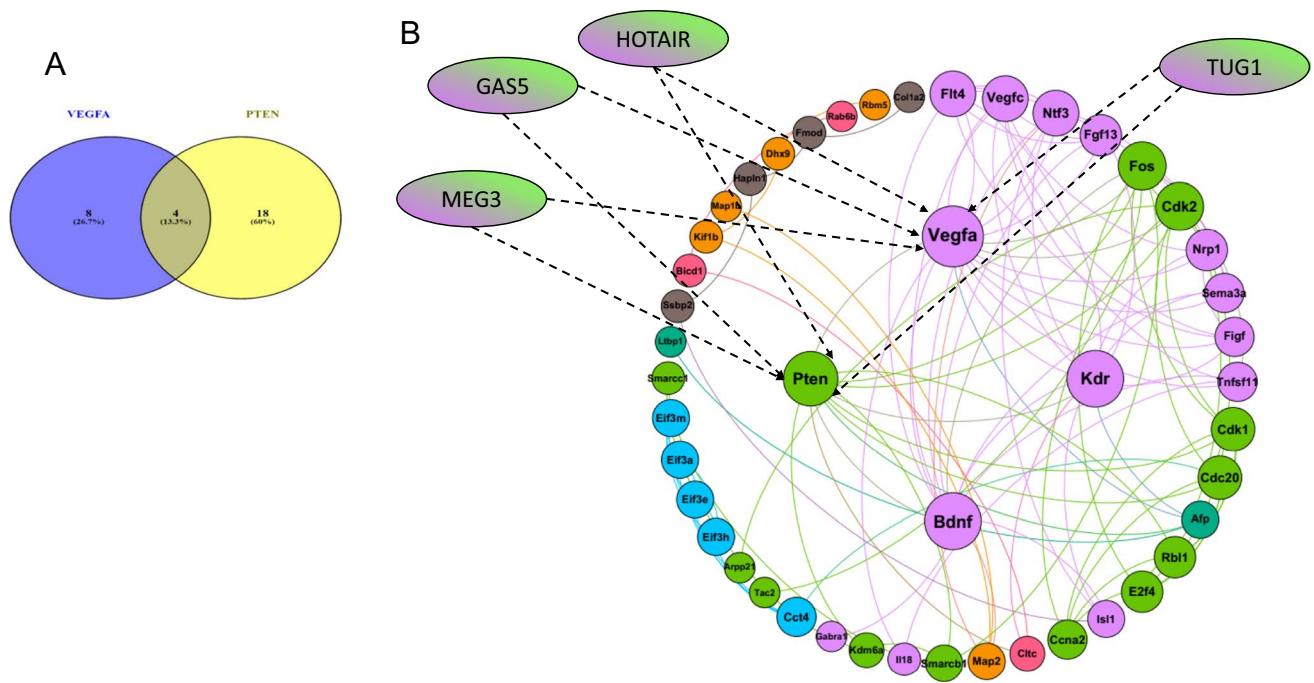
(s) with no target, total distance traveled (cm) with a target, and time spent in interaction zone (s) with the target were predominantly decreased compared with the control group (Table 4:  $P < 0.01$ ). Therefore, depression diminished mobility and induced social deficit-associated behavior based on these data.

### Physical Activity and the Leucine Complement Ameliorated the Brain and Gut Axis in Mice with Depression-Like Behaviors

Based on the in silico machine, we found that Kdr/Vegf $\alpha$ /Pten/Bdnf predicted a vital role in the brain and gut axis in mice with depression-like behaviors. Moreover, these molecules were common between the hippocampus region

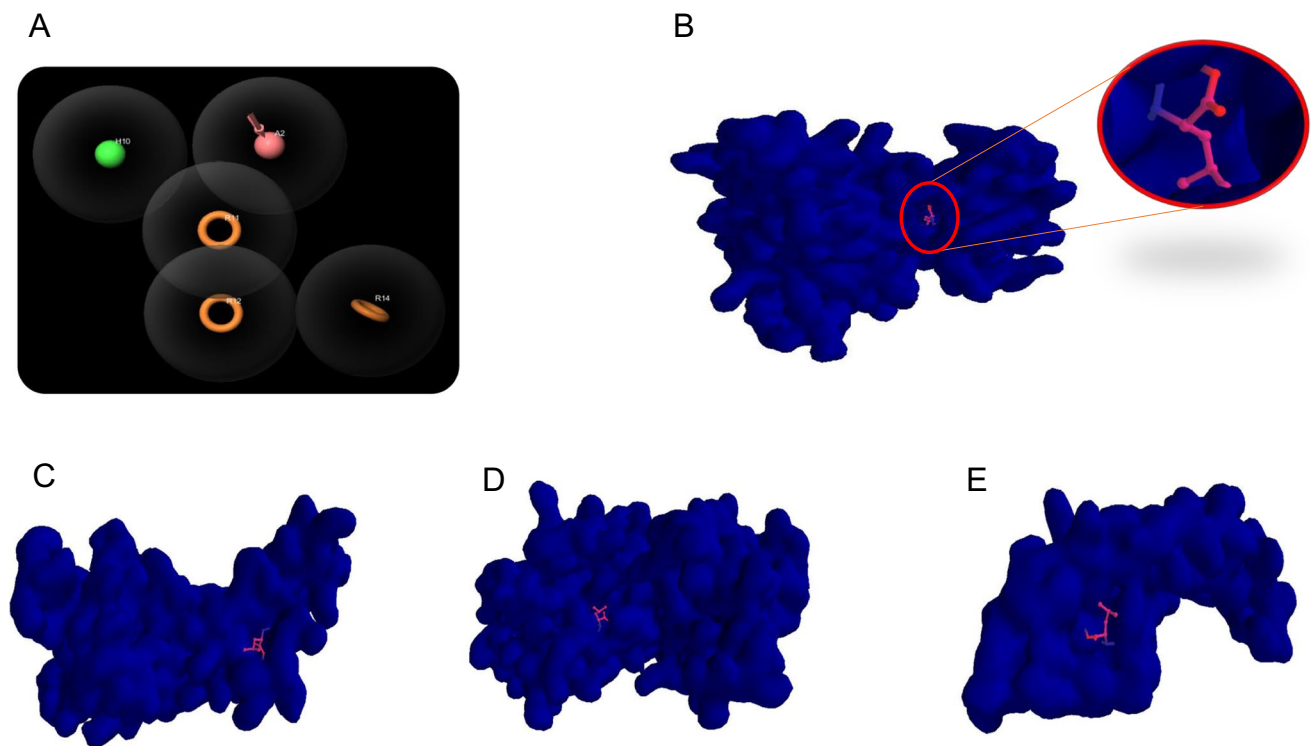
and ileum tissue. Therefore, we assessed the expression levels of Kdr/Vegf $\alpha$ /Pten/Bdnf in the hippocampus region and ileum tissue of mice with depression-like behaviors (Fig. 8A–H:  $P < 0.01$ ). Based on our results, we indicated that the relative expression of Pten in mice with depression-like behaviors significantly increased compared with the control group in the hippocampus region and ileum tissue (Fig. 8A, B:  $P < 0.01$ ). In addition, the data indicated that the expression level of the VEGF $\alpha$ /Kdr/Bdnf in the hippocampus region and ileum tissue was significantly reduced in mice with depression-like behaviors compared with the control group (Fig. 8C–H:  $P < 0.01$ ). In addition, exercise and leucine consumption decreased the relative expression of the Pten (Fig. 8A, B:  $P < 0.01$ ) and significantly enhanced the expression level of VEGF $\alpha$ /Kdr/Bdnf in the hippocampus region and ileum tissue (Fig. 8C–H:  $P < 0.01$ ). On the other hand, based on pharmacophore modeling and in silico analysis, KDR as a principal cutpoint protein in the network was identified. Our data found that KDR was a vital molecule in the Kdr/Pten/Vegf $\alpha$ /Bdnf network. Hence, we assessed the protein expression of the KDR in the hippocampus region (Fig. 8I:  $P < 0.01$ ). In this study, we indicated that the expression protein of KDR was significantly decreased in mice with depression-like behaviors compared with the control group (Fig. 8I:  $P < 0.01$ ). Also, exercise and the leucine complement enhanced the protein level of KDR (Fig. 8I:  $P < 0.01$ ). Notably, we observed the same pattern between protein and mRNA expression of KDR in the hippocampus region (Fig. 8F, I:  $P < 0.01$ ). Moreover, the protein level of PTEN significantly increased in mice with depression-like behaviors vs. the control group in the hippocampus region (Fig. 8J:  $P < 0.01$ ). Our data revealed that the level of PTEN protein was reduced by exercise and the leucine complement (Fig. 8J:  $P < 0.01$ ). Interestingly, the interaction of the leucine complement and exercise significantly decreased the level of PTEN protein (Fig. 8J:  $P < 0.01$ ). Based on this data, the leucine complement and physical activity could suppress the expression level of PTEN in the hippocampus region (Fig. 8J:  $P < 0.01$ ).

Interestingly, the interaction between exercise and the leucine complement significantly affected the Kdr/Vegf $\alpha$ /Pten/Bdnf signaling pathway (Fig. 8A–J:  $P < 0.01$ ). Furthermore, based on these results, we discovered that the interaction between exercise and the leucine complement had a synergistic effect on depression (Fig. 8A–J:  $P < 0.01$ ). As a result, physical activity and the leucine complement ameliorated the brain and gut axis in mice with depression-like behaviors. To confirm that physical activity and the leucine complement could improve the physical features of mice with depression-like behaviors, we evaluated the social deficit-associated behavior



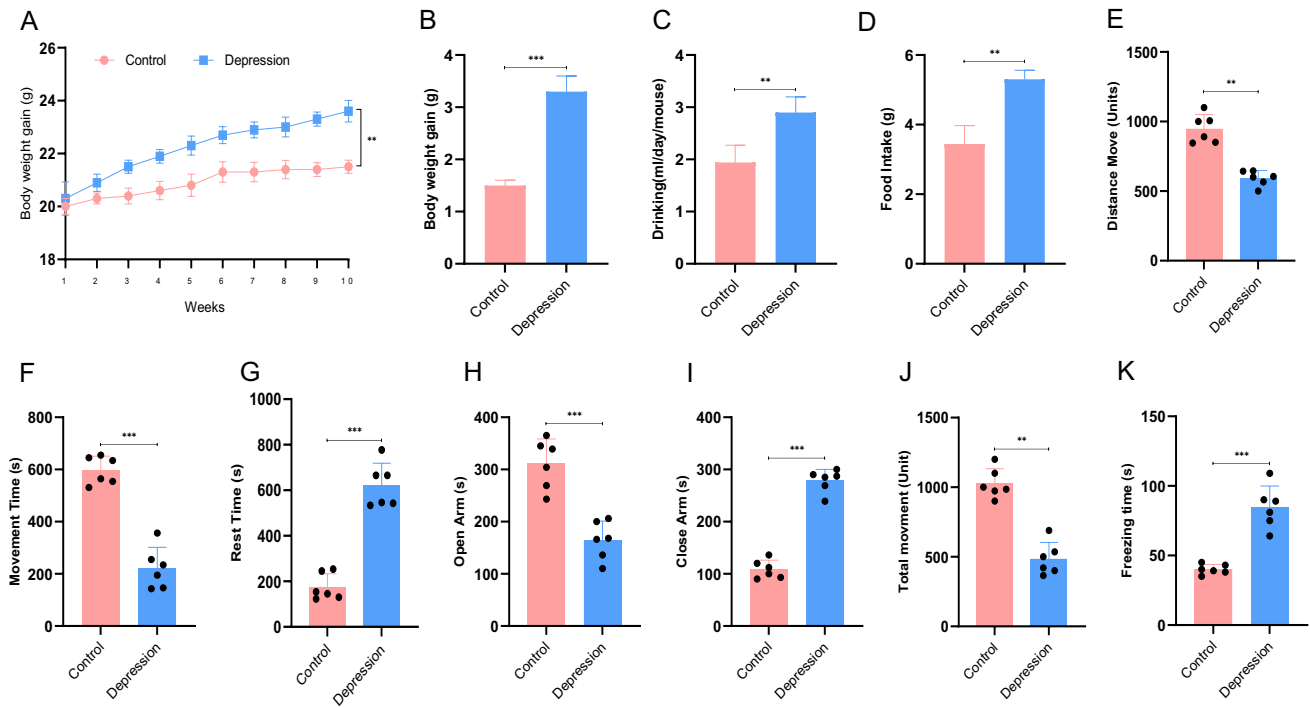
**Fig. 5** mRNA-lncRNA interaction network construction. **A** Common lncRNAs GAS5, HOTAIR, MEG3, and TUG1 are visualized in the intersection between the *Vegfa* and *Pten* in a circular diagram. **B**

Construction of interaction network between common hub proteins and selected lncRNAs among predicted lncRNAs list



**Fig. 6** Virtual screening for drug discovery. **A** The pharmacophore modeling based on 251 active ligands of Kdr ( $IC_{50} < 1$ ) included atomic features, Acceptor, hydrophobic, aromatic ring, aromatic ring, an aromatic ring with the survival score of 5.762731; the 3D structure of leucine did not align with the pharmacophore model built. **B**

The molecular docking method estimated the leucine complement's suitable and stable binding affinity on Kdr surface protein. **C–E** Non-suitable binding power between the leucine complement and BDNF, PTEN, and VEGF $\alpha$  proteins based on molecular docking outcomes could not influence the function of these proteins



**Fig. 7** Phenotype feature and behavior tests of depressed and control mice. **A** Weight gain was measured each week. (mean  $\pm$  SD;  $n=6$ ). **B** The difference in body weight gain between the first and last days of the experiment (g). (mean  $\pm$  SD;  $n=6$ ). **C** The food intake was calculated for the depressed and control mice based on daily consumption. (mean  $\pm$  SD;  $n=6$ ). **D** Weekly water consumption during the experiment. (mean  $\pm$  SD;  $n=6$ ). **E** Calculated distance moved (Unit) via open field test. (mean  $\pm$  SD;  $n=6$ ). **F** Movement time performance as measured in an open-field test based on seconds (s). (mean  $\pm$  SD;  $n=6$ ). **G** Calculated rest time (s). (mean  $\pm$  SD;  $n=6$ ). **H** Measured

open arms in plus elevated test based on seconds (s). (mean  $\pm$  SD;  $n=6$ ). **I** Measured closed arms in plus elevated test based on seconds (s). (mean  $\pm$  SD;  $n=6$ ). **J** Calculated total distance movement (Unit) via open-field test. (mean  $\pm$  SD;  $n=6$ ). Calculated freezing time. (mean  $\pm$  SD;  $n=6$ ). **\*\*** $P < .01$ , **\*\*\*** $P < .001$ . Data were analyzed by one-way analysis of variance (ANOVA) and Tukey’s post hoc test. It should be noted that for reducing the error in this study, the data of mice behavioral tests were obtained on triplicate datasets for each sample

**Table 4** Social interaction test

Behavior	Control	Depression
Total distance moved (cm) with no target	921 $\pm$ 92	632 $\pm$ 81**
Time spent in interaction zone (s) with no target	49 $\pm$ 8	21 $\pm$ 6**
Total distance traveled (cm) with target	862 $\pm$ 65	326 $\pm$ 39**
Time spent in interaction zone (s) with target	500 $\pm$ 45	95 $\pm$ 10**

\*\* Compared to the control group

(Fig. 8K–P:  $P < 0.01$ ). Hence, the open-fields test revealed that exercise and leucine elevated mobility and decreased rest time (Fig. 8K–M:  $P < 0.01$ ).

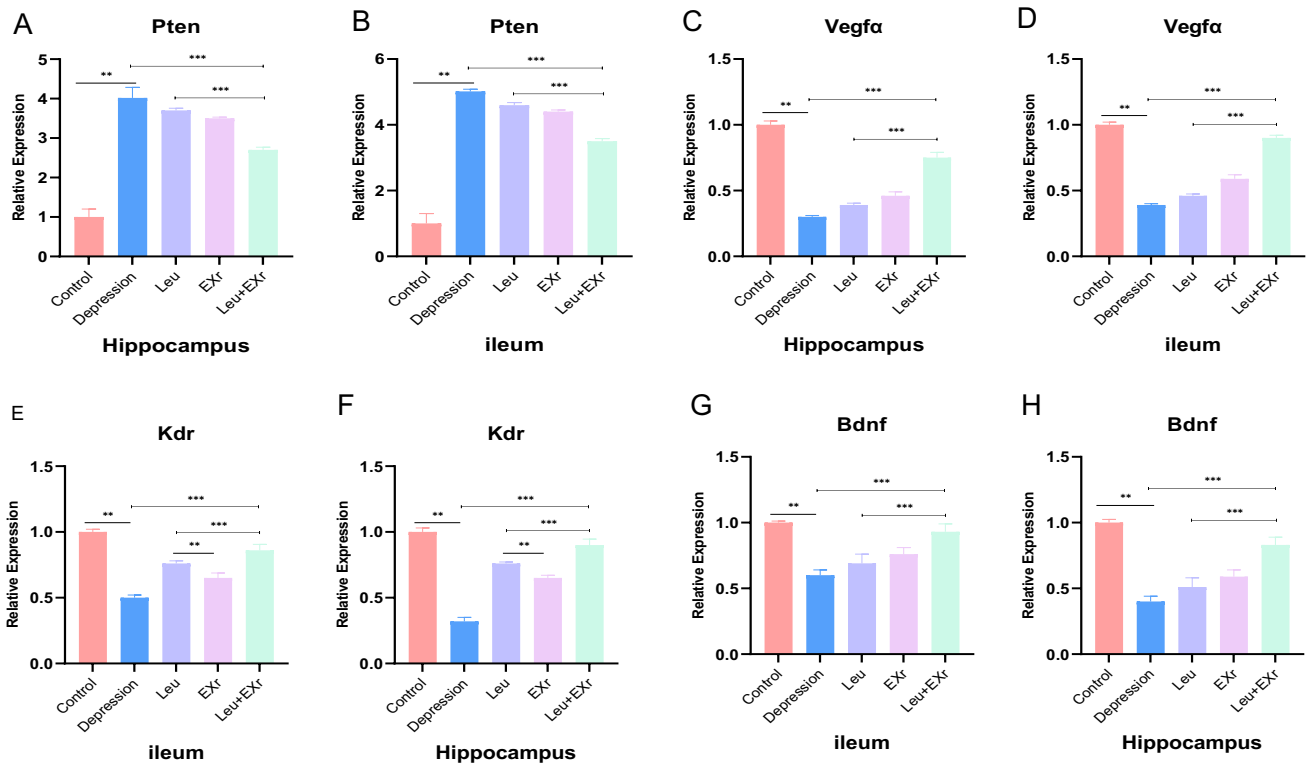
Moreover, the plus-maze test demonstrated that the number of closed and opened arms entries decreased and increased in the Leu, EXr, and Leu + EXr groups, respectively (Fig. 8N, O:  $P < 0.01$ ). Also, the whole movement of mice significantly increased in Leu, EXr, and Leu + EXr groups compared with the depression group (Fig. 8P:  $P < 0.01$ ). Therefore, we found that the physical activity and

leucine complement had a synergetic effect on the activity and sociability of the mice with depression-like behaviors.

### Exercise and Leucine Regulated the lncRNA in Mice with Depression-Like Behaviors

To validate the function of lncRNA prediction, we evaluated the relative expression of the four lncRNA associated with the *Kdr/Vegfa/Pten/Bdnf* in the hippocampus region and ileum tissue (Fig. 9A–H:  $P < 0.01$ ). Our data indicated that the expression level of HOTAIR was increased in the mice with depression-like behaviors in the hippocampus region and ileum tissue (Fig. 9A, B:  $P < 0.01$ ). Moreover, endurance exercise (EXr group) and leucine consumption (Leu group) were lower than in mice with depression-like behaviors in the hippocampus region and ileum tissue (Fig. 9A, B:  $P < 0.01$ ). In addition, we found the synergetic effect on the interaction between exercise and the leucine complement compared with other groups (Leu + EXr group). In addition, we demonstrated that the relative expression of the MEG3 and





**Fig. 8** Exercise and leucine complement improved the Kdr/Vegf $\alpha$ /Pten/Bdnf interaction network and depression condition. **A–H** The relative mRNA expression was measured by qRT-PCR for Pten, VEGF $\alpha$ , Kdr, and Bdnf transcripts relative to Gapdh in the hippocampus region and ileum tissue (mean  $\pm$  SD;  $n=6$ ). **I** The protein level of KDR/GAPDH (mean  $\pm$  SD;  $n=2$ ). **J** The protein level of PTEN/GAPDH (mean  $\pm$  SD;  $n=2$ ). **K** Measured distance move (Unit) via open field test. (mean  $\pm$  SD;  $n=6$ ). **L** Calculated the rest time (s).

(mean  $\pm$  SD;  $n=6$ ). **M** Movement time was measured in an open field test based on seconds (s). (mean  $\pm$  SD;  $n=6$ ). **N** Measured the open arms entrance (s). (mean  $\pm$  SD;  $n=6$ ). **O** Measured the closed arms entrance (s). (mean  $\pm$  SD;  $n=6$ ). **P** Measure total movement (Unit). (mean  $\pm$  SD;  $n=6$ ), \*\* $P < .01$ , \*\*\* $P < .001$ . Data were analyzed by one-way analysis of variance (ANOVA) and Tukey's post hoc test. It should be mentioned that in order to minimize error, data for this research were obtained in triplicate for each sample

GAS5 significantly decreased in mice with depression-like behaviors (Fig. 9C–F:  $P < 0.01$ ). Furthermore, exercise and the leucine complement were higher than in mice with depression-like behaviors, and both had synergistic effects on ameliorating depression (Fig. 9C–F:  $P < 0.01$ ).

Based on these data, we could conclude that HOTAIR had positive regulation as well as MEG3 and GAS5 lncRNAs had negative regulation with the Pten gene. Moreover, MEG3 and GAS5 lncRNAs had positive regulation with the Vegf $\alpha$  gene, and HOTAIR lncRNA had negative regulation with Vegf $\alpha$  in the hippocampus region and ileum tissue.

Notably, TUG1 lncRNA was significantly changed in the mice's hippocampus region and ileum tissue with depression-like behaviors compared with the control group (Fig. 9G, H:  $P < 0.01$ ). Furthermore, exercise and the leucine complement significantly enhanced the relative expression of the TUG1 in the hippocampus region (Fig. 9G:  $P < 0.01$ ). However, we found different patterns of the TUG1 level in

the ileum tissue (Fig. 9H:  $P < 0.01$ ). Our data indicated that the expression level of the TUG1 was enhanced in mice with depression-like behaviors in ileum tissue (Fig. 9H:  $P < 0.01$ ). Moreover, expression of the TUG1 predominantly declined with exercise and consumption of leucine (Fig. 9H:  $P < 0.01$ ). Hence, TUG1 lncRNA had different functions in the hippocampus region and ileum tissue. Moreover, based on these data, we might discover the lncRNA profile, which could play a vital role in depression and dysregulate these lncRNAs causing depression and exercise, and the leucine complement could regulate these lncRNAs and improve the depression pathomechanism.

## Discussion

In this study, we addressed the role of the brain-gut axis in mice with depression-like behaviors. Based on the in silico machine, we explored the pivotal hub genes and precise pathomechanism

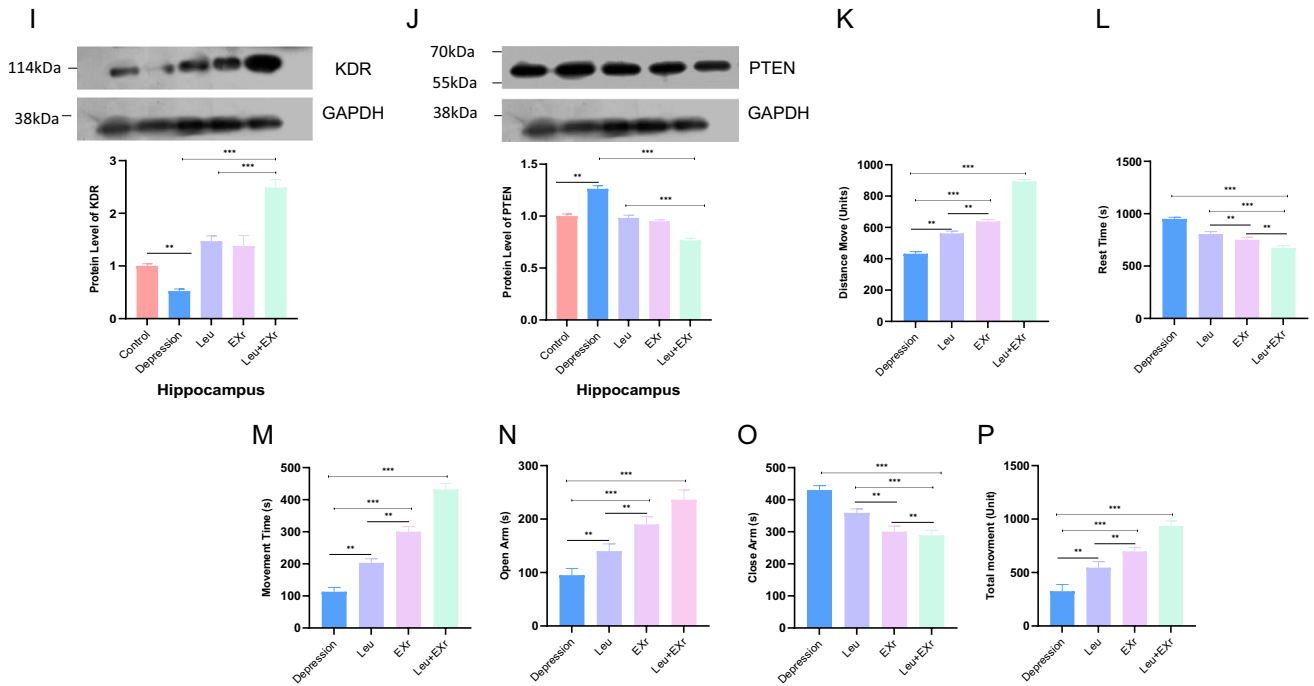


Fig. 8 (continued)

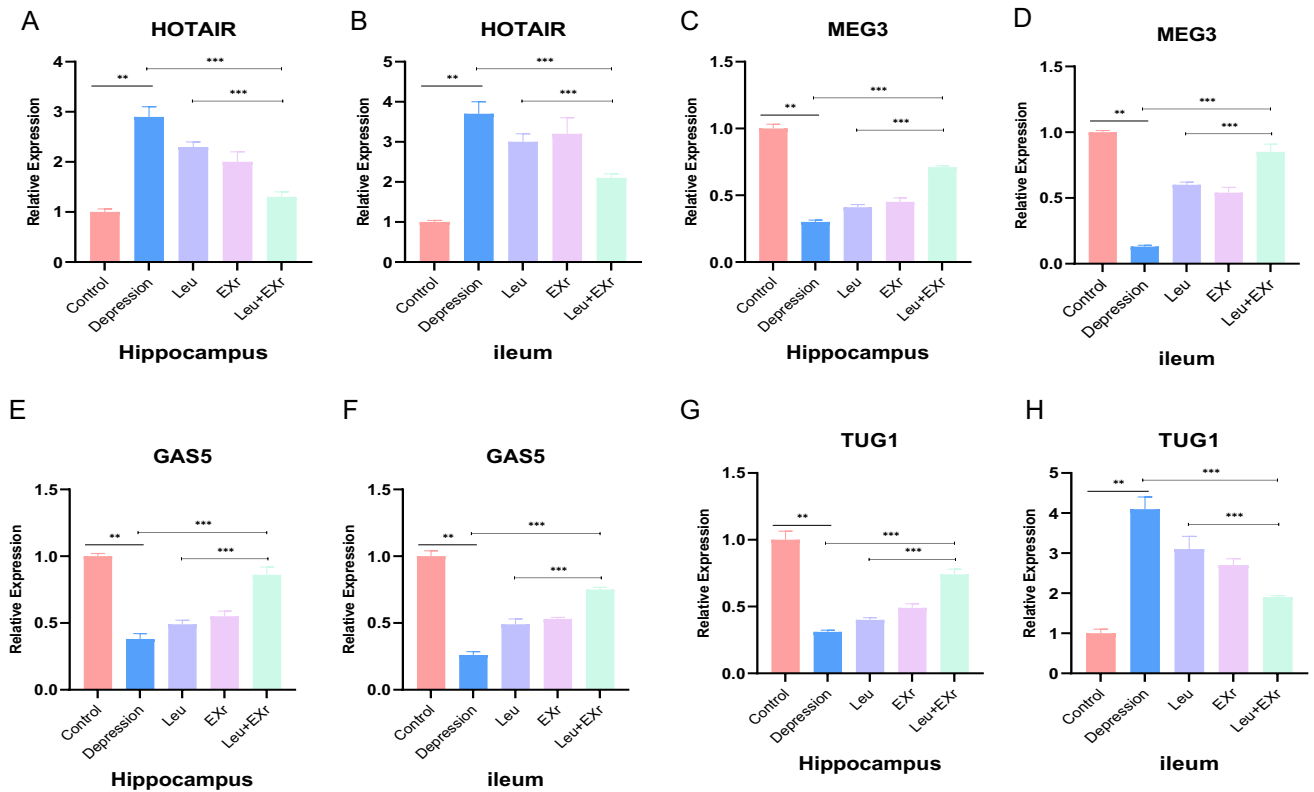


Fig. 9 Exercise and leucine complement modulated the lncRNA network. A–H The relative lncRNA expression was measured by qRT-PCR for HOTAIR, MEG, TUG1, and GAS5 transcripts relative to

GAPDH in the hippocampus region and ileum tissue (mean ± SD; n = 6). \*\*P < .01, \*\*\*P < .001. Data were analyzed by one-way analysis of variance (ANOVA) and Tukey’s post hoc test

in depression conditions. Moreover, we found communication between network mapping hub genes in the hippocampus region and ileum tissue via artificial intelligence. In addition, systems biology analysis indicated that various signaling and molecular mechanisms, including dopaminergic synapse, platelet activation, focal adhesion, Rap1 signaling pathway, Notch signaling pathway, cell cycle, AGE-RAGE signaling pathway, cytokine-cytokine receptor interaction signaling pathway, immunodeficiency, TNF signaling pathway, IL-17 pathway, Toll-Like receptor, and cell differentiation, are involved in the pathomechanism of the hippocampus region and ileum tissue in depression. Text mining of molecular signaling pathways indicated the role of these pathways in gut-brain pathogenesis based on *in silico* surveys and bioinformatics outcomes. Dopaminergic neurons, defined as the especially enteric nervous system, with the change of norepinephrine precursor (dopamine) to the active form of norepinephrine, could be regulated by both plexuses of bowel functions in the absence of central nervous system (CNS) input [51].

Moreover, many neurotransmitters launched in the CNS have also been found in the enteric nervous system [51]. On the other hand, platelet activation as the critical process has been associated with brain aging and neurodegenerative pathogenesis. The platelet activation process might trigger brain vascular abnormalities such as microvascular integrity disruption in depression conditions to preserve vascular integrity [52]. In a recent study, Ge Zhang and colleagues illustrated a disposition to platelet hyperactivity and microthrombosis risk in intestinal tissue in inflammatory bowel diseases, especially Crohn's [32]. Furthermore, growing evidence reported that AGE-receptor expression levels in neurodegenerative disorders were upregulated compared to normal subjects' samples [53]. These outcomes obtained from previous studies verified that RAGE is a facilitator for neuropathologic alteration [54, 55]. In the other study, Cristina Luceri et al. mentioned that the expression profile of advanced glycation end-products (AGEs) could increase the inflammation condition of intestinal tissue [56]. These results suggested that the AGEs-RAGE signaling pathway might be critical in inflammatory bowel pathogenesis. In fact, due to the multi-ligand receptor conditions for RAGE implicated in immune activation and inflammatory agent secretion, this signaling is identified as one of the potential therapeutic approaches in enteric inflammation [56]. Since the proliferation-differentiation and cell cycle programming are crucial in body homeostasis, Notch signaling pathways could be an effective cell fate determination in various tissues [57]. Therefore, Notch signaling pathways were disrupted in mice with depression-like behaviors in the hippocampus and gut tissues. As new results, we demonstrated that the Kdr/Vegf $\alpha$ /Pten/Bdnf interaction network could be decisive molecules in the brain-gut axis of mice with depression-like behaviors through bioinformatic analysis. In this present

study, we found that the relative expression of Kdr/Vegf $\alpha$ /Pten/Bdnf was dysregulated in major depressive conditions. Our data indicated that the expression level of the Pten was significantly increased in the brain and ileum tissues.

Wang et al. have reported that Pten could play an indispensable role in regulating depression in mice [58]. Furthermore, they indicated that Pten might be a mediator for neuronal atrophy and depression. This data was in agreement with our results. Moreover, Kdr, Vegf $\alpha$ , and Bdnf were reduced in the brain and ileum tissues in mice with depression-like behaviors. Furthermore, we evaluated the profile of lncRNAs that could regulate this interaction network. Based on system biology analysis, we explored that HOTAIR, MEG3, GAS5, and TUG1 lncRNAs were associated with Kdr/Vegf $\alpha$ /Pten/Bdnf interaction network and could be a principal modulator hippocampus region and ileum tissue of the depression condition. Interestingly, these lncRNAs profile could be a precise candidate and strategy to predict the prognosis and diagnosis of depressive disorder.

There is currently no effective treatment method for depression. Notably, only 60% of depression therapies effectively alleviated depressive symptoms [18]. Meanwhile, psychotherapy and antidepressant pills have been indicated to be effective in the therapeutic treatment of depression; not all are subjected to these treatments, and the effect sizes of treatments are generally modest [59]. Hence, several strategies are required to target prognosis and diagnosis or even halt psychiatric disorders [60]. One beneficial and convenient approach might consider exercise and physical activity [61]. Accordingly, physical activity and exercise training have been identified as helpful treatment techniques and mind-body practice in complementary and alternative health approaches [62]. Regular exercise training may significantly influence physical performance, cognitive improvement, metabolism, microbiota balance, neurohumoral adaptation, and hormone function on a long-term basis [55, 63].

Moreover, as a physiological and natural complement, leucine is a beneficial substance with various applications in cellular and molecular processes. This natural complement may be complementary and alternative medicine in preventing and alleviating illness hallmarks synchronized with medical prescription. Exercise is considered a non-pharmacological intervention that might be a valuable strategy to halt and manage the onset of depression [25, 26]. Evidence has been reported that exercise reduces the risk of depression [23, 24]. Hu et al. performed a merged systematic review and meta-analyses and indicated that exercise might benefit depression [61]. These results agreed with our data; we showed that physical activity improved the behavior tests related to depression and mobility in mice with depression-like behaviors.

Interestingly, leucine consumption could improve essential neurotransmitters and halt depression hallmarks.

Moreover, it was well-established that sufficient physical activity and essential nutrients might be requirements for producing neurotransmitters [22, 27–29]. In the cross-sectional study on 3175 individuals, consumption of branched-chain amino acids significantly reduced depression [64]. In addition, leucine is a beneficial substance with various applications in cellular and molecular processes. This natural complement may be complementary and alternative medicine in preventing and alleviating illness hallmarks synchronized with prescription medication. According to this evidence, leucine might be an effective and safe complement in treating several neurodegenerative disorders and psychological disorders. In addition, the relative expression of the mRNA-lncRNA network correlation with depression condition was modulated by endurance exercise.

Interestingly, based on pharmacophore modeling and molecular docking surveys, we indicated that KDR as the initiator element among four selected hub proteins could be a strong candidate for the depressive therapeutic approach and improve the gut-brain axis function. Although the leucine complement was not aligned with KDR's pharmacophore model, computing binding power illustrated that supplementation with suitable and stable binding affinity on the KDR protein surface positively affected brain and intestine functions. Furthermore, we demonstrated that consumption of the leucine complement regulated the *Kdr/Vegfa/Pten/Bdnf* interaction network and HOTAIR, MEG3, GAS5, and TUG1 lncRNAs.

Sideromenos and colleagues indicated that VEGF could modulate depression-related behavior and be the candidate antidepressant agent [65]. In addition, they found that treatment based on VEGF stimulated the phosphorylation of ERK in the hippocampus. Notably, our study revealed that exercise and leucine complement increased the relative expression of *Vegfa* mRNA and MEG3 and GAS5 lncRNAs. Based on our data, MEG3 and GAS5 had positive regulation related to the *Vegfa* gene, and HOTAIR lncRNA negatively correlated with *Vegfa* in the brain and ileum tissues. Thus, we could conclude that consumption of leucine and endurance exercise might have a synergetic effect on regulating the *Kdr/Vegfa/Pten/Bdnf* interaction network and lncRNAs.

One of the limitations of our study was the lack of data on protein expression of the *Vegfa* and *Bdnf*. Moreover, we did not consider the exercise training with various intensities, repetitions, and duration for selecting the most efficient exercise program in depression therapy. We suggest that physical activity and leucine consumption in both genders can investigate in the following studies.

## Conclusion

Here, according to artificial intelligence, biological analysis, and pharmaceutical approaches for major depressive amelioration, we could conclude that synchronizing leucine

consumption and regular endurance exercise might have a synergetic effect on the predicted regulatory network containing hub proteins, *Kdr/Vegfa/Pten/Bdnf* interaction network, and network long non-coding RNAs in the improved gut-brain axis.

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**Author Contribution** N. A. and F. T. did the experiments; design of the study was performed by N. A. and analyses by F.H.B. N.A. performed data mining, and F.H.B. interpreted and obtained information. Technical assistance was done by N. A. and F.H.B. N.A wrote the manuscript. F.H.B. was approved by F.T.

**Availability of data and materials** The data and materials that support the findings of this study are available from the corresponding author upon reasonable request.

## Declarations

**Ethics Approval** Approval of mouse usage in this study was obtained by the Ethics committee of Isfahan (Khorasgan) Branch of Islamic Azad University (IR.IAU.KHUISF.REC.1400.160).

**Consent to Participate** Not applicable.

**Consent for Publication** All authors support submission to this journal.

**Conflict of Interest** The authors declare no competing interests.

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